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Methicillin-Resistant Coagulase-Negative Staphylococci on Pig Farms as a Reservoir of Heterogeneous Staphylococcal Cassette Chromosome *mec* Elements

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Methicillin-resistant *Staphylococcus aureus* (MRSA) likely originated by acquisition of the staphylococcal cassette chromosome *mec* (SCC*mec*) from coagulase-negative staphylococci (CNS). However, it is unknown whether the same SCC*mec* types are present in MRSA and CNS that reside in the same niche. Here we describe a study to determine the presence of a potential *mecA* reservoir among CNS recovered from 10 pig farms. The 44 strains belonged to 10 different *Staphylococcus* species. All *S. aureus* strains belonged to sequence type 398 (ST398), with SCC*mec* types V and IVa. Type IVc, as well as types III and VI, novel subtypes of type IV, and not-typeable types, were found in CNS. *S. aureus*, *S. epidermidis*, and *S. haemolyticus* shared SCC*mec* type V. The presence of SCC*mec* type IVc in several staphylococcal species isolated from one pig farm is noteworthy, suggesting exchange of this SCC*mec* type in CNS, but the general distribution of this SCC*mec* type still has to be established. In conclusion, this study shows that SCC*mec* types among staphylococcal species on pig farms are heterogeneous. On two farms, more than one recovered staphylococcal species harbored the same SCC*mec* type. We conclude that staphylococci on pig farms act as a reservoir of heterogeneous SCC*mec* elements. These staphylococci may act as a source for transfer of SCC*mec* to *S. aureus*.

Globally, methicillin-resistant *Staphylococcus aureus* (MRSA), an important pathogen in humans and animals, is responsible for considerable mortality, morbidity, and health care expenditure in both hospitals and the community (29). Methicillin resistance is associated with the presence of the *mecA* gene, which encodes an additional penicillin-binding protein (PBP2a or PBP2′). This protein has a lower affinity for all beta-lactam antibiotics (12). The *mecA* gene is located on a mobile genetic element called staphylococcal cassette chromosome *mec* (SCC*mec*) (14).

The origin of SCC*mec* remains unknown, but it is believed that the *mecA* gene itself originated with one common precursor. Homologues of a *mecA* gene have been found in *Staphylococcus sciuri* (5) and *Staphylococcus vitulinus* (22). However, these *mecA* gene homologues are not located in a *mecA* complex as with SCC*mec*. Tsubakishita et al. (24) showed that a *mecA* gene homologue present in *Staphylococcus fleurettii* showed 99 to 100% sequence homology with the *mecA* gene present in MRSA strain N315. Additional sequence analysis showed the presence of an almost identical structure of the *mecA* complex. This result indicates that a direct precursor of the methicillin resistance determinant for MRSA is present in *S. fleurettii*, which is a member of the *S. sciuri* group within the staphylococci (24). *S. fleurettii* is a commensal bacterium of animals, and having the ancestor of the *mecA* gene present in an animal-borne staphylococcal species suggests that SCC*mec* elements may be generated in a *Staphylococcus* species that has an animal as its normal host. The possibility that *mecA* may have originated with *S. fleurettii* strengthens the hypothesis that MRSA probably acquired SCC*mec* from coagulase-negative staphylococci (CNS) (11). This hypothesis is further supported by the fact that methicillin resistance among human clinical isolates is more prevalent in CNS than in *S. aureus* (6, 17). Furthermore, the observation of *in vivo* transfer of SCC*mec* from *Staphylococcus epidermidis* to *S. aureus* (31) suggests that CNS may act as a source for SCC*mec* acquisition by *S. aureus*. This would be consistent with the finding that SCC*mec* types present in CNS are more heterogeneous than those in MRSA (11, 18, 32).

Worldwide methicillin-resistant CNS (MRCNS) have been isolated from a number of animals, such as pigs, horses, cows, dogs, and cats (8, 29, 30). Recently, MRSA belonging to sequence type 398 (ST398) emerged in livestock (pigs, veal calves, and poultry) in Europe and North America (10, 23, 30), whereas in Asia, livestock-associated MRSA belonging to ST9 emerged (28). Contact with animals colonized with MRSA has been recognized as a risk factor for human colonization (9, 25). The increasing number of MRSA ST398 transmissions and infections illustrates that this is a public health concern (29).

Although it has been proposed that methicillin-susceptible *S. aureus* (MSSA) ST398, highly prevalent in pigs, acquired *mecA* from coexisting staphylococci (30), the presence of *mecA*-positive staphylococci in pigs has not been studied and the source of SCC*mec* in MRSA ST398 is only speculative. Therefore, it was the aim of this study to detect *Staphylococcus* species harboring SCC*mec* on pig farms. We demonstrate that a reservoir of *mecA*-positive CNS coincides with *S. aureus* on pig farms and that the presence of the same SCC*mec* types among CNS and *S. aureus* supports the hypothesis that on pig farms CNS may act as a reservoir for exchange of SCC*mec*.

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MATERIALS AND METHODS

Study design. This study included 10 pig farms in The Netherlands with different levels of antibiotic usage, which is expressed in animal daily dosages per year. Nasal samples were taken from 5 randomly selected pigs, using dry cotton swabs. In addition, on each farm 5 dust samples were taken using dry cotton swabs. The swabs were immediately transported to the laboratory and processed within 24 h after collection.

The collection of swabs from pigs was approved by the Animal Experimental Committee of Utrecht University according to the Dutch Law on Animal Health and Welfare.

Staphylococcal isolation. The swabs from each farm were pooled in two samples, one with the nasal swabs and one with the dust swabs. Material was eluted in 5 ml phosphate-buffered saline (PBS) by vigorous shaking and vortexing for 1 min. One hundred microliters of the obtained sample suspension was inoculated on mannitol-salt agar (bioTRADING, The Netherlands) using 10-fold serial dilutions up to 10^{-4} and incubated for 72 h at 30°C. An additional 100 μl of undiluted sample suspension was inoculated on Brilliance Staph 24 agar (Oxoid, United Kingdom), an MRSA selective plate, and incubated for 24 h at 37°C. From each mannitol-salt agar plate, colonies with typical but diverse staphylococcal morphologies were selected and cultivated on blood agar with 5% sheep blood (bioTRADING, The Netherlands) for further analysis (to a maximum of 5 isolates that displayed the same morphology). Moreover, from the Brilliance Staph 24 agar, up to 5 typical MRSA colonies were selected and subcultured for further analysis. After incubation for 24 h at 37°C on blood agar with 5% sheep blood (bioTRADING, The Netherlands), staphylococcal strains were verified by colony morphology, Gram staining, and catalase reaction.

DNA isolation. From each isolate, crude DNA was isolated using InstaGene matrix (Bio-Rad, The Netherlands) according to the protocol of the manufacturer.

Identification of *mecA*-positive staphylococci. Detection of the *mecA* gene was carried out by multiplex PCR using primers for *mecA* (7) and the 16S rRNA gene (27F and 556R) (15) using amplification of the 16S rRNA gene as a positive control for DNA extraction. PCR was carried out in a 20-μl volume containing 1× PCR master mix (MBI Fermentas, Lithuania) with 6 μM *mecA* primers, 3 μM 16S rRNA gene primers, and 2 μl of crude DNA. The thermal cycling conditions used were 95°C for 5 min followed by 35 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min, and the final extension was at 72°C for 5 min. PCR products were detected on a 1.5% agarose gel stained with ethidium bromide.

The diversity of *mecA*-positive isolates was assessed by GTG-fingerprinting PCR analysis according to the protocol of Braem et al., with modifications (3). Briefly, the amplification mixture consisted of a total volume of 25 μl containing 1× PCR buffer (MBI Fermentas, Lithuania), 1.25 mM $MgCl_2$, 0.4 mM deoxynucleoside triphosphates (dNTPs), 10% dimethyl sulfoxide (DMSO), 200 μg bovine serum albumin (BSA), 50 pmol primer and 2 U *Taq* polymerase (MBI Fermentas, Lithuania), and 2 μl DNA solution. Amplifications were carried out using reported amplification conditions (3). The resulting fingerprints were analyzed using the BioNumerics V 6.0 software package (Applied Maths, Ghent, Belgium). The similarity among digitized profiles was calculated using Pearson's correlation and the unweighted pair group method with arithmetic means (UPGMA) with 2% optimization. Isolates recovered from the same source exhibiting distinct GTG-fingerprinting profiles (similarities less than 95%) were considered to be genetically unrelated and were included for further analysis.

Species identification of selected isolates was performed using matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) according to the protocol of the manufacturer (Bruker, Bremen, Germany). Raw spectra were analyzed by the MALDI Biotyper 2.0 software program (Bruker Daltonics) with default settings. An internal control (*Escherichia coli* DH5α) was used for calibration before each experiment. Identification scores above 2 or between 1.8 and 2 for duplicate samples were considered to be reliable (4). In isolates with unreliable identification (below 1.8), species determination was performed by sequencing of 16S rRNA genes (15) and *tuf* genes (21). Sequences were analyzed against all available sequences using the BLAST algorithm. The species was identified when the gene sequences yielded ≥98% sequence similarity with the closest bacterial species sequence in GenBank.

SCC*mec* typing. The SCC*mec* elements were typed by a recommended hierarchical system (13). This included four multiplex PCRs (M-PCRs) according to a protocol reported previously by Kondo et al. (16). In addition, where a noninterpretable type was obtained by M-PCR, a single PCR of each gene was performed. When the *ccr* complex and *mecA* complex could not be amplified, no SCC*mec* type was assigned. For subtyping of the SCC*mec* type IVa isolates, the *ccrB* allotype was determined by *ccrB* sequence typing (20). Positive controls for the M-PCR were the MRSA strains COL/SCC*mec* type I, N315/SCC*mec* type II, ANS46c/SCC*mec* type III, MW2/SCC*mec* type IVa, and S217/SCC*mec* type V (clinical isolate from the University Medical Center Utrecht, Utrecht, The Netherlands).

Rapid *S. aureus* ST398 identification. All *S. aureus* were screened for ST398 using a real-time assay based on the detection of the C01 amplified fragment length polymorphism (AFLP) region (27). PCR was carried out in a 20-μl volume containing 1× LightCycler 480 probe master (Roche, Germany) with 0.9 μM CO1 primers (27) and 0.2 μM CO1 probe (5′ Yakima Yellow-ATTGTCAGTATGAATTGCGGT-MGB 3′) and 2 μl of DNA matrix. The real-time PCR cycling conditions used were 95°C for 10 min followed by 45 cycles of 95°C for 10 s, 58°C for 20 s, and 72°C for 20 s, and cooling was at 40°C for 10 s. Real-time PCR amplification was carried out with a LightCycler 480 instrument (Roche, Germany).

Nucleotide sequence accession numbers. The *ccrB* sequences of the two pig isolates from farm 5, classified as new alleles 417 and 418, have been deposited in www.ccrbtyping.net as isolates F0101-01 and F0101-12, respectively.

RESULTS

Staphylococci recovered from pig farms. A total of 65 *mecA*-positive staphylococci were isolated from 7 of the 10 pig farms. From farms 1, 2, and 6, no *mecA*-positive staphylococci were isolated (Table 1). All analyzed staphylococcal isolates were typeable using GTG fingerprinting (Fig. 1). Isolates recovered from the same source exhibiting distinct GTG-fingerprinting profiles (similarities less than 95%) were considered to be genetically unrelated and were included for further analysis: in total, 44 isolates. Of these 44 *mecA*-positive staphylococci, 33 (75%) were isolated from nose swabs and 11 (25%) from dust samples. For each isolate, the species could be determined. The most common species recovered from the nasal samples were *S. aureus* ($n = 15$; isolated from pigs on 4 farms), *Staphylococcus cohnii* ($n = 6$; isolated from pigs on 1 farm), *Staphylococcus haemolyticus* ($n = 4$; isolated from pigs on 2 farms), and *S. epidermidis* ($n = 3$; from pigs on 1 farm). Single isolates of *S. sciuri*, *Staphylococcus pasteuri*, *Staphylococcus equorum*, *Staphylococcus saprophyticus*, *Staphylococcus lentus*, and *S. fleurettii* were recovered from nasal swabs. The staphylococci isolated from the dust on two farms belonged to *S. aureus* ($n = 4$ from 2 farms) and *S. epidermidis* ($n = 2$ from 2 farms). Also, single isolates of *S. haemolyticus*, *S. saprophyticus*, *S. cohnii*, *S. sciuri*, and *S. equorum* were isolated from the dust samples.

Species distribution differed between farms. In general, a higher number of *mecA*-positive isolates were recovered from farms with high antibiotic usage (Table 1). In addition, on farms with high antibiotic usage, a larger number of different *mecA*-positive *Staphylococcus* species also were isolated (Table 1).

GTG-PCR fingerprinting. All analyzed staphylococcal isolates were typeable using GTG fingerprinting. The generated PCR amplicons ranged in size from 200 bp to 8 kb, and the number of PCR products ranged from 4 to 24 bands. GTG fingerprinting showed

TABLE 1 Characterization of *mecA*-positive staphylococci[a]

Farm	Staphylococcal species isolated from pig nostril (n^b)	SCC*mec* type(s)	Staphylococcal species isolated from dust (n^b)	SCC*mec* type	No. of ADD/yr	Total no. of isolates
1	None		None		<5	0
2	None		None		<5	0
3	*S. equorum* (1)	ND	None		<5	1
4	*S. lentus* (1)	ND	None		<5	1
5	*S. aureus* (2)	IVa	*S. aureus* (1)	IVa	11.2	5
	S. pasteuri (1)	IVc				
	S. haemolyticus (1)	V				
6	None		None		13.1	0
7	*S. fleurettii* (1)	ND	None		21	1
8	*S. aureus* (4)	V	*S. saprophyticus* (1)	IV	30	5
9	*S. aureus* (3)	V	*S. equorum* (1)	VI	32	7
	S. epidermidis (2)	V, ND	*S. epidermidis* (1)	V		
10	*S. aureus* (6)	V	*S. aureus* (3)	V	35	24
	S. cohnii (6)	IVc	*S. cohnii* (1)	IVc		
	S. sciuri (1)	III	*S. sciuri* (1)	ND		
	S. haemolyticus (3)	IVc, IVvar	*S. haemolyticus* (1)	IVvar		
	S. saprophyticus (1)	IVc	*S. epidermidis* (1)	IVc		
Total no. of isolates	33		11			44

[a] ADD/yr, animal daily dosages per year (antibiotic usage); ND, not determined; var, unknown subtype variant.
[b] *n*, no. of isolates.

a high diversity of GTG patterns among the recovered staphylococcal isolates (Fig. 1). The GTG fingerprints grouped the individual species (*S. aureus*, *S. cohnii*, *S. haemolyticus*, *S. epidermidis*, and *S. equorum*) in separate clusters, except for one *S. aureus* isolate. Isolates of *S. cohnii*, which were all isolated from farm X, were located on 3 different positions in the dendrogram. The single isolates obtained for *S. fleuretti*, *S. pasteuri*, and *S. lentus* clustered separately. The GTG fingerprints of isolates recovered from dust samples and pigs from the same farm clustered together.

Diversity of SCC*mec* types in staphylococci. All selected isolates were screened for SCC*mec* types using a multiplex strategy. In 36 of 44 isolates, known SCC*mec* types were detected: V ($n = 19$), IVc ($n = 12$), IVa ($n = 3$), III ($n = 1$), and VI ($n = 1$). In the remaining 8 isolates, we were not able to detect the exact SCC*mec* types by using the M-PCR strategy. In 3 isolates, the SCC*mec* types belonged to a new variant of type IV, which could not be subtyped using the Kondo M-PCR strategy. In 5 isolates, the SCC*mec* types were nontypeable. Table 1 lists the detected SCC*mec* types in recovered isolates, and Table 2 shows the nontypeable SCC*mec* elements.

SCC*mec* type V was predominant on the investigated farms. This type was harbored mostly by *S. aureus* isolates ($n = 16/19$), which were present on 3 farms. Additionally, SCC*mec* type V was found in two *S. epidermidis* isolates and one *S. haemolyticus* isolate. Furthermore, only on farm 9, SCC*mec* type V was present in two different species: *S. epidermidis* and *S. aureus*. SCC*mec* type IVc, the second predominant type, was identified in 12 isolates recovered from 2 farms (farms 5 and 10). On farm 10, 4 different species harbored SCC*mec* IVc ($n = 11$): *S. cohnii* ($n = 7$; pig and dust samples), *S. haemolyticus* ($n = 1$; pig), *S. pasteuri* ($n = 1$; pig), *S. saprophyticus* ($n = 1$; pig), and *S. epidermidis* ($n = 1$; dust). SCC*mec* type IVa was identified only in *S. aureus* isolates recovered from farm 5, and SCC*mec* types III and VI were detected in single isolates.

In 3 *S. haemolyticus* isolates recovered from farm X, the SCC*mec* type was defined as type IV based on the presence of the *ccrA2B2* genes and *mecA* complex B. However, subtyping based on amplification of the J1 region showed the presence of open reading frame (ORF) E007 in two isolates, which corresponds to SCC*mec* type I, and for the other *S. haemolyticus* isolate, subtyping was unsuccessful. These isolates, we concluded, were carrying a new variant of SCC*mec* type IV (IVvar) (Table 1).

The SCC*mec* type of 5 isolates, recovered from 5 different farms, could not be determined (Table 2). In 4 isolates, only the presence of a *mecA* complex B or A could be determined. Moreover, in one *mecA*-positive *S. epidermidis* isolate, neither the presence of the *ccr* genes nor that of the *mecA* complex could be determined.

To exclude possible transfer of *S. aureus* SCC*mec* type IVa from the community, we performed *ccrB* sequence typing. The *ccrB* sequences of the two pig isolates from farm 5 were closely related to *ccrB2* allele 401 from a human *S. aureus* isolate in the database and were classified as new alleles 417 and 418. This indicates the presence of new *ccrB2* alleles, which until now have not been found in humans but were associated with the pig farm environment.

DISCUSSION

Horizontal gene transfer of the SCC*mec* element is thought to contribute to the generation of new methicillin-resistant staphylococci, including MRSA. To investigate a potential site of such an exchange of SCC*mec* elements, our study focused on the presence of SCC*mec* among the staphylococcal flora present on 10 selected pig farms.

In this study, 10 different methicillin-resistant *Staphylococcus* species were recovered from 7 pig farms. The most common species was *S. aureus*, but the majority of the isolates were CNS. Typing of SCC*mec* from MRCNS has been described for only one

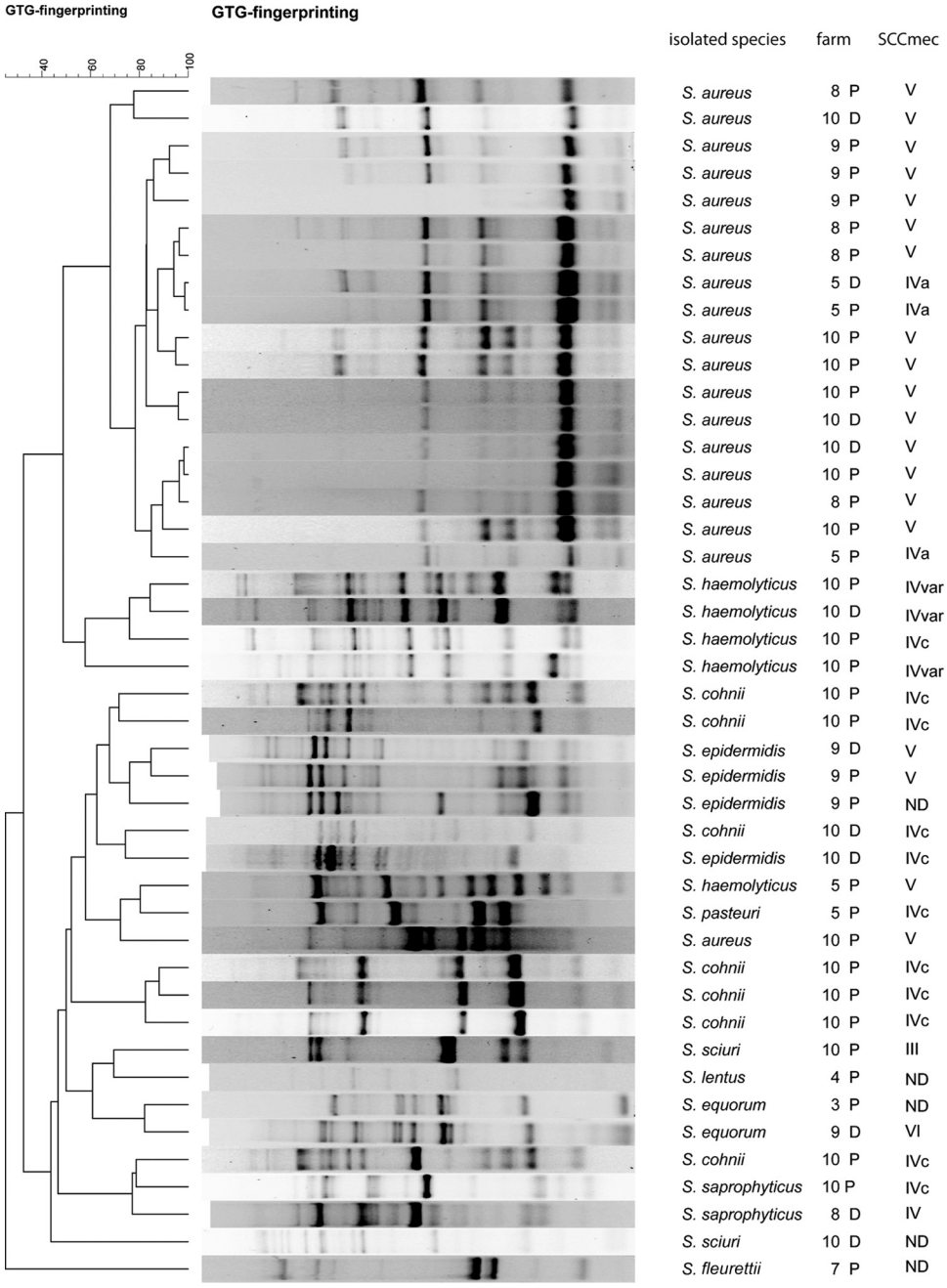

FIG 1 Dendrogram based on the cluster analysis of the $(GTG)_5$-PCR fingerprinting profiles of recovered *mecA*-positive staphylococci in this study using UPGMA clustering methods of Pearson's correlation coefficients. P, isolate recovered from pig nose; D, isolate recovered from dust.

other study conducted in pigs. However, in that study, only carriage of *S. sciuri* with SCC*mec* type III was described, and methicillin-resistant *S. lentus* and *S. xylosus* were isolated but their SCC*mec* elements were not typed (32).

From the pig nose and dust samples, the same *Staphylococcus* species were obtained. Typing of the isolates from pig nose and dust samples showed that they were genetically related and suggests transmission of staphylococci between dust and the pig nose. The fact that multiple species were isolated from the pig nose suggests that colonization of methicillin-resistant *Staphylococcus* species occurs in the nose, which may create the environment for potential horizontal gene transfer.

All *S. aureus* isolates belonged to ST398 (1, 23). Also, GTG fingerprinting could not differentiate the isolates and supports the clonal spread of MRSA ST398 in pig farms. Remarkably, the species distributions and numbers of recovered *mecA*-positive staphylococci were different on the investigated farms. In general, a higher number of methicillin-resistant isolates were recovered from farms with high antibiotic usage, and also a larger number of different methicillin-resistant *Staphylococcus* species were iso-

TABLE 2 Characterization of nontypeable SCC*mec* carried by recovered CNS

Staphylococcal species (farm no./sample type[a])	Presence of:		*mecA* complex[b]
	mecA	*ccr* genes[b]	
S. equorum (3/P)	+	−	B
S. lentus (4/P)	+	−	B
S. fleurettii (7/P)	+	−	B
S. sciuri (10/D)	+	−	A
S. epidermidis (9/P)	+	−	ND[c]

[a] P, pig; D, dust.
[b] As determined by Kondo PCR (16).
[c] ND, not determined.

lated. In the recovered staphylococci, SCC*mec* typing showed the carriage of known types (III, IVa, IVc, V, and VI). In 3 isolates, new subtypes of SCC*mec* type IV were found, and in 5 isolates, we were unable to identify the SCC*mec* element. In the recovered MRSA, we found only SCC*mec* types V and IVa, which are commonly identified SCC*mec* types for MRSA ST398 (23); the presence of two different SCC*mec* types in *S. aureus* ST398 with the same *spa* type (data not shown) suggests, as has been shown previously (26), that different SCC*mec* types have been transferred to an isogenic MSSA isolate. How often this transfer occurs is unknown, but the farm environment, where multiple *mecA*-positive staphylococci reside, may provide an environment for potential lateral transfer. Another finding of a possible reservoir in pig farms is the finding of *S. aureus* harboring novel SCC*mec* variants as defined by *ccrB* sequencing. The detection of novel *ccrB2* alleles, which have not been found in human isolates until now, suggests the possibility that these *S. aureus* strains were not introduced by human contact.

Our results indicate a large diversity in the J1 region in type IV of SCC*mec* in CNS. For example, *S. cohnii*, *S. haemolyticus*, *S. saprophyticus*, and *S. pasteuri* SCC*mec* type IVc was detected; in two *S. haemolyticus* isolates, sequences associated with SCC*mec* type I in SCC*mec* subtype IV were found; and in one *S. haemolyticus* isolate, SCC*mec* type IV could not be subtyped, indicating the presence of novel SCC*mec* subtypes. This is consistent with the findings of Berglund et al. (2), who reported new types of SCC*mec* type IV based on variation in the J1 region in MRSA. Our results indicate that on pig farms, type IV and type V are the major SCC*mec* types in the staphylococcal population. SCC*mec* type VI, harbored by one isolate of *S. equorum*, has not been detected in this species before. We detected SCC*mec* type III only in one isolate of *S. sciuri*, which was previously reported by Zhang et al. (32). A SCC*mec* element that was nontypeable with the Kondo PCRs was found in one *S. sciuri* isolate. SCC*mec* typing was furthermore impossible in single isolates of *S. epidermidis*, *S. equorum*, *S. lentus*, and *S. fleurettii*. These findings indicate the presence of novel SCC*mec* elements in CNS isolates, are in agreement with findings of other studies (11, 18, 32), and show that diversity of SCC*mec* types in CNS is considerably larger than that in MRSA recovered from the same environment.

MRCNS are considered to be a source for horizontal gene transfer of SCC*mec* elements to *S. aureus*. The diversity of SCC*mec* types among CNS is larger than that among *S. aureus* (6, 17), and interspecies horizontal transfer of SCC*mec* from *S. epidermidis* to *S. aureus* has recently been documented (31). Moreover, as the study by Nübel et al. indicated, transfer of SCC*mec* to *S. aureus* is not a rare event (19). All of these observations support the hypothesis that CNS may act as the SCC*mec* reservoir for interspecies exchange of this element among *Staphylococcus* species. Our results are also consistent with this hypothesis, since the *S. aureus* and *S. epidermidis* isolates shared the SCC*mec* V element and because of the presence of SCC*mec* type IVc in four staphylococcal species. Our study showed a large diversity of SCC*mec* among the staphylococci present in pig farms, which may be a risk for interspecies lateral transfer of SCC*mec* between CNS and *S. aureus*. Detailed genetic characterization of the SCC*mec* types in these strains is necessary to confirm that exactly the same SCC*mec* elements are present in these *Staphylococcus* species.

The novel SCC*mec* types found in this study, as well as type IVc, have not previously been found in MRSA ST398. Transfer of these subtypes to *S. aureus*, generating novel MRSA strains, cannot be excluded, and longitudinal surveillance will be required to follow dissemination of novel SCC*mec* types in *S. aureus* ST398 or other sequence types.

A limitation of our study is that only 10 farms were included. This did not allow us to draw conclusions about the relationship between antibiotic use and the number of different methicillin-resistant staphylococcus species or the bacterial load. Furthermore, additional SCC*mec* types, both known and novel, may be present in the farm environment while not being detected because of the limited number of farms included.

In conclusion, SCC*mec* elements present in staphylococci on pig farms are highly heterogeneous. These staphylococci may act as a source for transfer of SCC*mec* to *S. aureus*. Although direct proof of transfer was not obtained in this study, SCC*mec* type V was shared in *S. aureus* and *S. epidermidis* and SCC*mec* type IVc was present in 4 MRCNS, indicating the possibility of interspecies transfer of SCC*mec* elements in pig farms.

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